$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and
f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Resolution.* Calculate the resolution (*R*) as follows:

$$R = \frac{2(t_{Rj} - t_{Ri})}{w_i + w_j}$$

where:

t_{Rj}=Retention time of *t*-butyl ceftazidime;
t_{Ri}=Retention time of pyridine;
w_i=Width of pyridine peak at the baseline measured by extrapolating the relatively straight sides to the baseline; and
w_j=Width of *t*-butyl ceftazidime peak at the baseline measured by extrapolating the relatively straight sides to the baseline.

(3) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation for the pyridine peak (*S_R* in percent) as follows:

$$S_R = \frac{100}{\overline{X}}\left[\frac{\sum\limits_{i=1}^{n}\left(X_i - \overline{X}\right)^2}{N-1}\right]^{1/2}$$

where:

$\overline{X}$ is the mean of *N* individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, except alternate injections of the working standard solution with injections of the sample solution.

[50 FR 48398, Nov. 25, 1985; 50 FR 53308, Dec. 31, 1985]

§ 436.360 Gel permeation chromatographic assay for high molecular weight polymer.

(a) *Equipment.* A suitable gel permeation chromatograph equipped with.

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 50 centimeters long and 9 millimeters internal diameter, packed with a material as defined in the monograph for the drug being tested.

(b) *Procedure.* Perform the assay and calculate the high molecular weight polymer content using the temperature, instrumental conditions, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 10 percent of scale with a typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of mobile phase for not less than 18 hours, removing any voids that may form at the top of the column, followed by five replicate injections of the same volume (100 microliters) of the blue dextran system suitability test solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (*T*), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and
f=Horizontal distance from point of ascent to

a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (*n*) of the column as follows:

$$n = 5.545 \left(\frac{t_R}{w_h} \right)^2$$

where:

n=Efficiency, as number of theoretical plates for column;
t_R=Retention time of solute; and
w_h=Peak width at half-height.

(3) *Coefficient of variation (relative standard deviation).* Calculate the coefficient of variation (S_R in percent) as follows:

$$S_R = \frac{100}{\overline{X}} \left[\frac{\sum\limits_{i=1}^{n} \left(X_i - \overline{X} \right)^2}{N-1} \right]^{1/2}$$

where:

$\overline{X}$ is the mean of *N* individual measurements of X_i.

If the complete operating system meets the system suitability requirements of the monograph for the drug being tested, proceed as described in paragraph (b) of this section, using two injections of the same volume (100 microliters) of the working standard solution followed by one injection of the same volume (100 microliters) of the sample solution.

[50 FR 48398, Nov. 25, 1985; 51 FR 1367, Jan. 13, 1986]

§ 436.361 High-performance liquid chromatographic assay for aztreonam.

(a) *Equipment.* A suitable high-performance liquid chromatograph equipped with:

(1) A suitable detection system specified in the monograph for the drug being tested;

(2) A suitable recording device of at least 25-centimeter deflection;

(3) A suitable chromatographic data managing system; and

(4) An analytical column, 3 to 30 centimeters long, packed with a material as defined in the monograph for the drug being tested; and if specified in that monograph, the inlet of this column may be connected to a guard column, 3 to 5 centimeters in length, packed with the same material of 40 to 60 micrometers particle size.

(b) *Procedure.* Perform the assay and calculate the drug content using the temperature, instrumental conditions, flow rate, and calculations specified in the monograph for the drug being tested. Use a detector sensitivity setting that gives a peak height for the working standard that is at least 50 percent of scale with typical chart speed of not less than 2.5 millimeters per minute. Use the equipment described in paragraph (a) of this section. Use the reagents, working standard solution, and sample solution described in the monograph for the drug being tested. Equilibrate and condition the column by passage of 10 to 15 void volumes of mobile phase followed by five replicate injections of the same volume (between 10 and 20 microliters) of the working standard solution. Allow an operating time sufficiently long to obtain satisfactory separation and elution of the expected components after each injection. Record the peak responses and calculate the prescribed system suitability requirements described for the system suitability test in paragraph (c) of this section.

(c) *System suitability test.* Select the system suitability requirements specified in the monograph for the drug being tested. Then, using the equipment and procedure described in this section, test the chromatographic system for assay as follows:

(1) *Tailing factor.* Calculate the tailing factor (*T*), from distances measured along the horizontal line at 5 percent of the peak height above the baseline, as follows:

$$T = \frac{W_{0.05}}{2f}$$

where:

$W_{0.05}$=Width of peak at 5 percent height; and
f=Horizontal distance from point of ascent to a point coincident with maximum peak height.

(2) *Efficiency of the column.* Calculate the number of theoretical plates (*n*) of the column as follows: